The Anatomy and Histology of *Phyllidia pulitzeri* Pruvot-Fol, 1962, with Remarks on the Three Mediterranean Species of *Phyllidia* (Nudibranchia, Doridacea)

by

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Abstract. In this study is given the first extensive examination of the anatomy and the histology of the organ systems of a phyllidiid: the Mediterranean species *Phyllidia pulitzeri* Pruvot-Fol, 1962.

The digestive tract of *Phyllidia pulitzeri* differs from that of other species of the genus by the lack of the typical oral glands lying on the outside of the oral tube. Oral glands can be found only at the inside wall of the oral tube. The genital system corresponds to the triaulic scheme of all other Doridacea. The penis has no armament. Gastro-esophageal ganglia, as described by other authors, are not present. Special features of the excretory system are the very long renopericardial duct and a glandular mass that lies in the posterior part of the nephridium and continues in the ureter. Basophilic subepithelial glands that are scattered over the mantle, foot, and gills seem to be responsible for the intensive secretion of mucus.

A comparison of the three Mediterranean species of *Phyllidia* (*P. rolandiae, P. aurata,* and *P. pulitzeri*) led to the conclusion that *P. rolandiae* is a *nomen dubium,* because its description does not allow a clear distinction from the two other species.
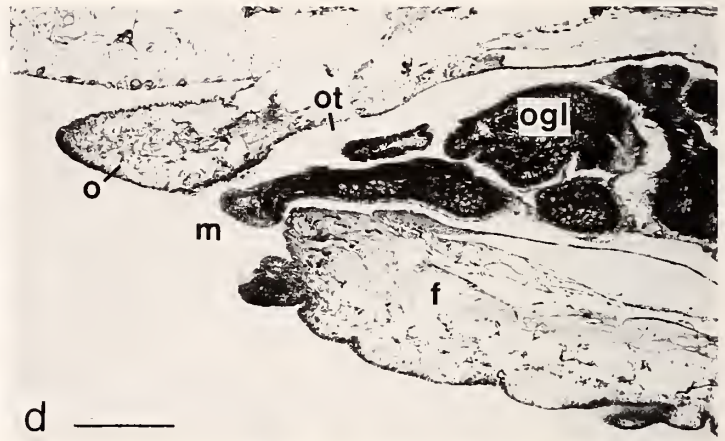
INTRODUCTION

"THE PHYLLIDIIDAE ARE the longest known of all nudibranchs (Bergh, 1876) and were figured as early as 1735" (MARCUS, 1962:477). Nevertheless, little is known about their anatomy and even less about the phylogeny of this family or the life histories of its species. It is occasionally maintained in the literature that dissection of phyllidian species is unnecessary for identification (*e.g.*, PRUVOT-FOL, 1962). Therefore, several species are known only by their external features. That dissection is an absolute necessity is demonstrated by several studies (EALES, 1938; ELIOT, 1903, 1904; MARCUS, 1962) which show that there are both interspecific and intraspecific differences.

The phyllidiids have an oval, flat form similar to the cryptobranchiate Doridacea, but they can be easily distinguished from those by their gills lying ventrally between the notum and foot, being interrupted only by the mouth and the genital papilla. The anus lies dorso-median (*Phyl-*

lidia, Phyllidiella, Phyllidiopsis, Ceratophyllidia) or ventro-median between the gill leaflets (*Fryeria*). The phyllidiids have no radula or mandible, a feature that they have in common with the Dendrodorididae and on which was based their association with the latter family into the group Porostomata (BERGH, 1876, after BERGH, 1889).

The most important anatomical feature for systematics is the foregut, as it distinguishes the two genera *Phyllidia* and *Phyllidiopsis*. Yet, the first description of *Phyllidia pulitzeri* Pruvot-Fol, 1962, is based on a single specimen only, and its internal anatomy is completely unknown. As a consequence its generic status is not certain. Does it belong to the genus *Phyllidia* or to *Phyllidiopsis*?

Having a few specimens in well-fixed condition at my disposal, I have been able to answer this question and to give a detailed description of the anatomy and the histology of some of the organs. Moreover, this examination is regarded as a first step toward a complete systematic re-

vision of the family Phyllidiidae and toward a determination of its correct phylogenetic position.

MATERIAL AND METHODS

In September 1980, four specimens of *Phyllidia pulitzeri* were collected by SCUBA diving in Khalkidhiki (Northern Aegean Sea/Greece). The animals were found on a sponge of the genus *Axinella* in depths between 10 and 20 m (Figure 1a). In May 1983, additional specimens were discovered in Gozo/Malta in depths between 5 and 30 m. They were collected from crevices, and a group of five animals was found on rock material at the back of a large cave.

The material from Greece (length of specimens: 22–32 mm; breadth: 12–22 mm) was fixed in 4% formaldehyde/seawater, that from Malta (length: 12.5–22 mm; breadth: 6–13 mm) in Bouin's fluid. Previous narcosis with MS 222 prevented deformation of the animals during fixation.

For anatomical and histological examinations, three specimens were embedded in paraplast. Serial longitudinal sections (8 μm) were made of two small animals (specimen L1: 12.5 mm; specimen L2: 22 mm) and serial cross sections (8 μm) of a larger specimen (C1: 31 mm). Staining of the sections was carried out according to ROMEIS (1968) with May-Grünwald/Giemsa or trichrome (azan or hemalaun/lightgreen). Four specimens were dissected under the stereomicroscope.

As the fixatives did not penetrate the digestive gland, histological examination of this organ was not possible.

The following descriptions apply to all specimens examined, except where special reference is made to particular specimens.

One specimen is deposited as a neotypus in the Muséum National d'Histoire Naturelle, Paris.

RESULTS

External Morphology

In the living animal the notum above the visceral hump is covered with white tubercles arranged in five longitudinal lines. Between them are smaller, orange-colored tubercles. On the margin of the notum the white and orange-colored warts are scattered irregularly (Figure 1b). Immediately behind the yellow rhinophores a small white tubercle is located on each rhinophore sheath (Figure 2a:

rhst), the so-called "Rhinophorenscheidentuberkel" (SCHMEKEL & PORTMANN, 1982:140).

Fixed animals are ivory-colored and the arrangement of the white tubercles in longitudinal lines is hardly visible. The tubercles of different animals vary in size and form. In some they are higher than broad and stand close together (Figure 2d). In others the warts are flat, widely separate, and protrude only slightly. This great variability in the form of the tubercles is due to fixation, because it was not observed in living animals.

The rhinophores, each with approximately 12–14 lamellae, are situated in the anterior ¼ to ⅕ of the body (Figure 1b). The rhinophore sheaths are very small. The anal papilla lies in an anal tubercle of varying size located medio-dorsally in the posterior ⅕ of the body (Figure 2b).

The ventral side of the living animal is uniformly gold to light yellow in color. The margin of the notum is slightly transparent and the radial and net-shaped arrangement of the spiculae is visible. Beneath the slitlike oral aperture, which is surrounded by two short, triangular, marginally grooved, oral tentacles, the foot has a short, longitudinal notch (Figure 2e). The 100–130 gill leaflets, lying in a groove between the foot and mantle margin, are triangular-shaped and are fused to the ventral notum along their broad base. Large and small gill leaflets alternate more or less regularly (Figure 2c).

The genital papilla lies on the right side in the anterior third of the body between the gill leaflets. The vestibulum (v), with vagina and vas deferens, opens on the distal part of the papilla and the oviduct (ov, Figure 2c) opens on the proximal part.

All the specimens fixed in formaldehyde are covered with small crystalline globules (Figure 1e). Some of them could easily be detached, but the greater part of them could not be removed without the surrounding tissue.

Digestive Tract

Anatomy: The general anatomical outline can be seen in Figure 2f. The vertical slit of the oral aperture leads into a vestibulum, from where the oral tube rises. After entering the periviscenal cavity (pc) the oral tube widens into a club-shaped chamber; the highly folded walls of this chamber consist of a glandular epithelium (ogl). There are no glands on the outside of the oral tube.

Two thick retractor muscles arise laterally, one on each

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Figure 1

a. Living animal in natural surrounding, on a sponge of the genus *Axinella*. Photograph was taken in Corfou/Greece in August 1974. Specimen was not collected; depth 10 m. b. Living animal from Gozo/Malta; note the transparency of the mantle margin. c. Longitudinal section through statocyst; arrows, neurilemma; May-Grünwald/Giemsa; scale 10 μm. d. Longitudinal section of the anterior digestive tract; note the protruding parts of the oral gland; azan; scale 50 μm. e. "Crystallized" mucus on the ventral side of the mantle margin; scale 10 μm. Key: f, foot; gnc, giant nerve cell; m, mouth; o, oral tentacle; ogl, "oral gland"; ot, oral tube; st, statolith.
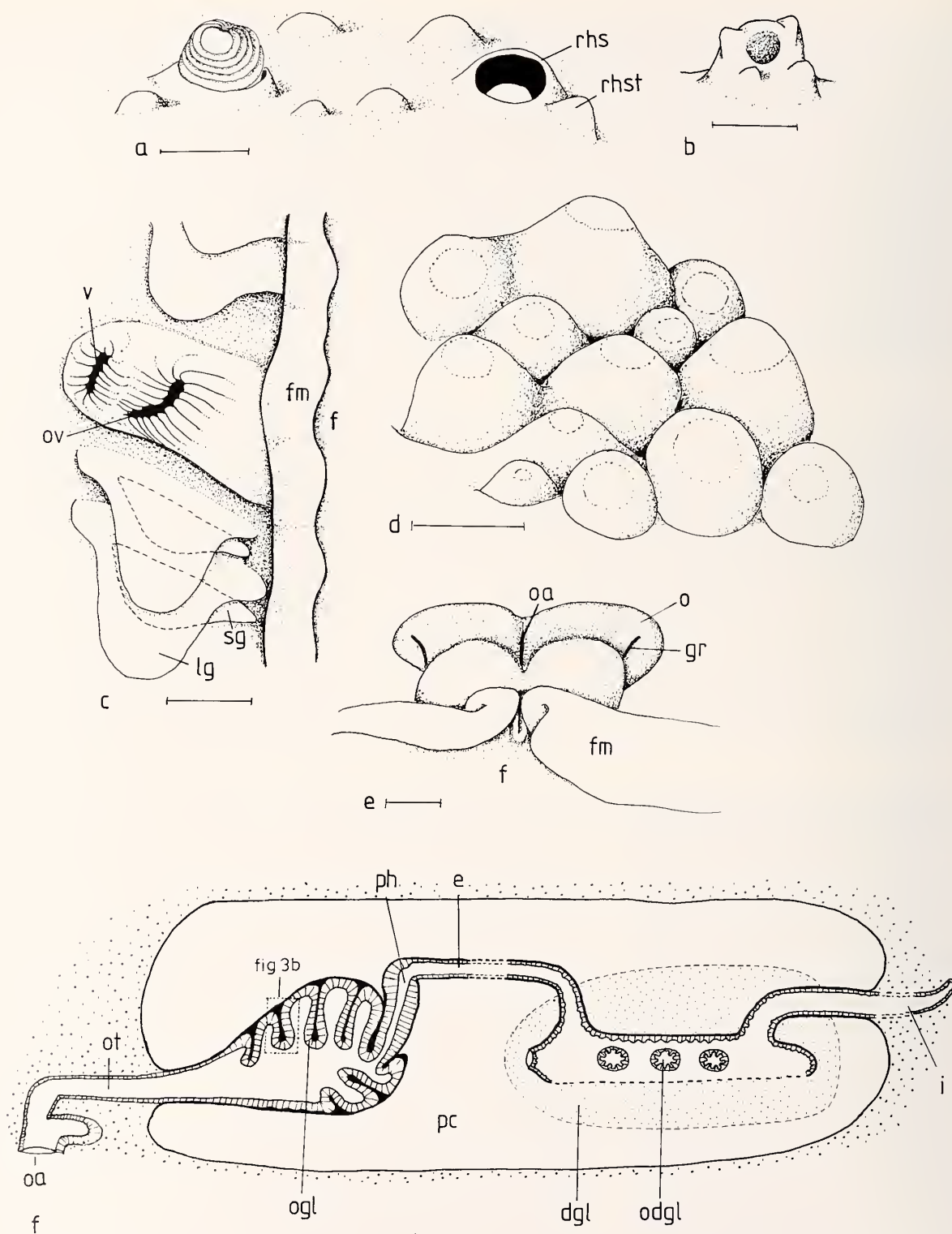
Figure 2

a–e. External morphology, details; scales 1 mm. a. Openings of the rhinophoral cavities. b. Anal papilla. c. Ventral side; genital papilla and gill leaflets (after WÄGELE, 1984). d. Tubercles of the fixed animal; they are high and stand very close together. e. Ventral side; oral tentacles (o). f. General outline of the digestive tract, longitudinal

side of the oral tube. The muscles run caudo-medially, inserting in the dorsal body wall. The pharynx arises between these two retractor muscles, narrows after two small bends into the esophagus and then passes through the central nerve ring.

The esophagus leads directly backward and opens into the stomach (Figure 3a).

A distinct separation between the stomach and a central lumen of the digestive gland is not present: several tubes of the digestive gland open laterally into a central cavity of the digestive tract (Figure 2f: odgl), where also the esophagus ends and the intestine starts dorsally.

The digestive gland occupies nearly ⅔ of the whole visceral hump. The ventral side is covered with a wide-spread network of vessels, which open into four visceral sinuses (see WÄGELE, 1984).

The intestine originates in the posterior third of the stomach, runs forward, and turns to the left, making an arch in front of the heart (Figure 3a: i). It then runs posteriorly on the right side under the pericardium and ends in the anal papilla.

Histology: Around the vestibulum and along the sides of the mouth lies a thick glandular mass; these glands open into the vestibulum or externally at the base of the oral tentacles.

The anterior oral tube is surrounded by connective tissue containing longitudinal muscular fibers laterally, and mainly transverse muscular fibers dorsally and ventrally. Cilia could not be detected by light microscopy. Goblet cells filled with basophilic grana are interspersed between the epithelial cells. The anterior oral tube seems to be very distensible: in one specimen, the oral glandular folds of the posterior oral tube (see below) were protruded out of the mouth, and the anterior oral tube was several times wider than in other specimens (Figure 1d).

The oral glands are situated inside of the posterior, club-shaped part of the oral tube (Figure 2f), the ectodermal epithelium of which projects inward, forming the folded and finger-shaped glands. The glandular folds (Figure 3b) contain, in particular, transversal and longitudinal muscle fibers. The ectodermal epithelium consists mainly of tall columnar cells, with the nuclei lying basally. Goblet cells similar to those of the oral tube are interspersed between them. Toward the apex of the glandular folds these mucus-secreting cells replace the epithelial cells. Subepithelial basophilic glands (bsgl) were recognized along the ectodermal epithelium. In the apex, additional granular glands with acidophilic contents (agl) could be seen in the connective tissue. However, it was not possible to decide whether these glands were single glandular cells or multicellular glands.

Most fibers of each of the great retractor muscles insert in one glandular septum of the corresponding side. Other branches envelop the posterior part of the oral tube and the anterior part of the pharynx. The pharynx consists (from outside to inside) of a thin layer of connective tissue (ct), a thin layer of circular muscles (cm), a layer of fewer radial muscle fibers (rm), and a thin layer of longitudinal muscle fibers (lm). An apocrine-secreting epithelium lines the pharyngeal lumen, which in cross section has the shape of a triangle. The inner layer of longitudinal muscle fibers is well developed along the sides of the triangle (Figure 3c).

The transition into the esophagus is abrupt. The esophagus is enclosed by a thin outer layer of connective tissue, followed by a layer of circular and a thick layer of longitudinal muscle fibers. The lumen is lined by smooth columnar epithelium (Figure 3d). Cilia could not be found by light microscopy. In the layer of circular muscles two nerves were observed (Figure 3d: nv).

The entrance of the esophagus into the stomach is characterized by regular folds of the, here, ciliated cuboidal epithelium. These folds continue in the dorsal wall of the stomach. Glandular cells were not detected. Ciliated epithelial linings exist only in dorsal areas around the openings of the esophagus and intestine, and sometimes around the large entrances into the digestive gland. The ventral epithelium is similar to that of the digestive gland.

The intestine is characterized by a cuboidal ciliated epithelium (Figure 7c), which in the proximal part is highly folded and invested by muscle fibers.

Genital Apparatus

Anatomy: The structure of the genital system of *Phyllidia pulitzeri* corresponds to that of all Doridacea: it is triaulic and bears two vesicles (bursa copulatrix and receptaculum seminis) on the vaginal duct (Figure 4a).

The hermaphroditic gonad is a more or less flat organ surrounded by the kidney dorsally and laterally and the digestive gland ventrally. Running initially for a short distance along the esophagus, the hermaphroditic duct (gonoduct gd) opens into a yellow-whitish oval ampulla (Figures 4b, c: a). The postampullar gonoduct (pogd) leads ventrally along the female gland, then divides into the vas deferens and oviduct.

After a short distance the vas deferens enlarges into the brownish prostate (pr) which, after a few bends, leads above the female gland directly to the right side of the
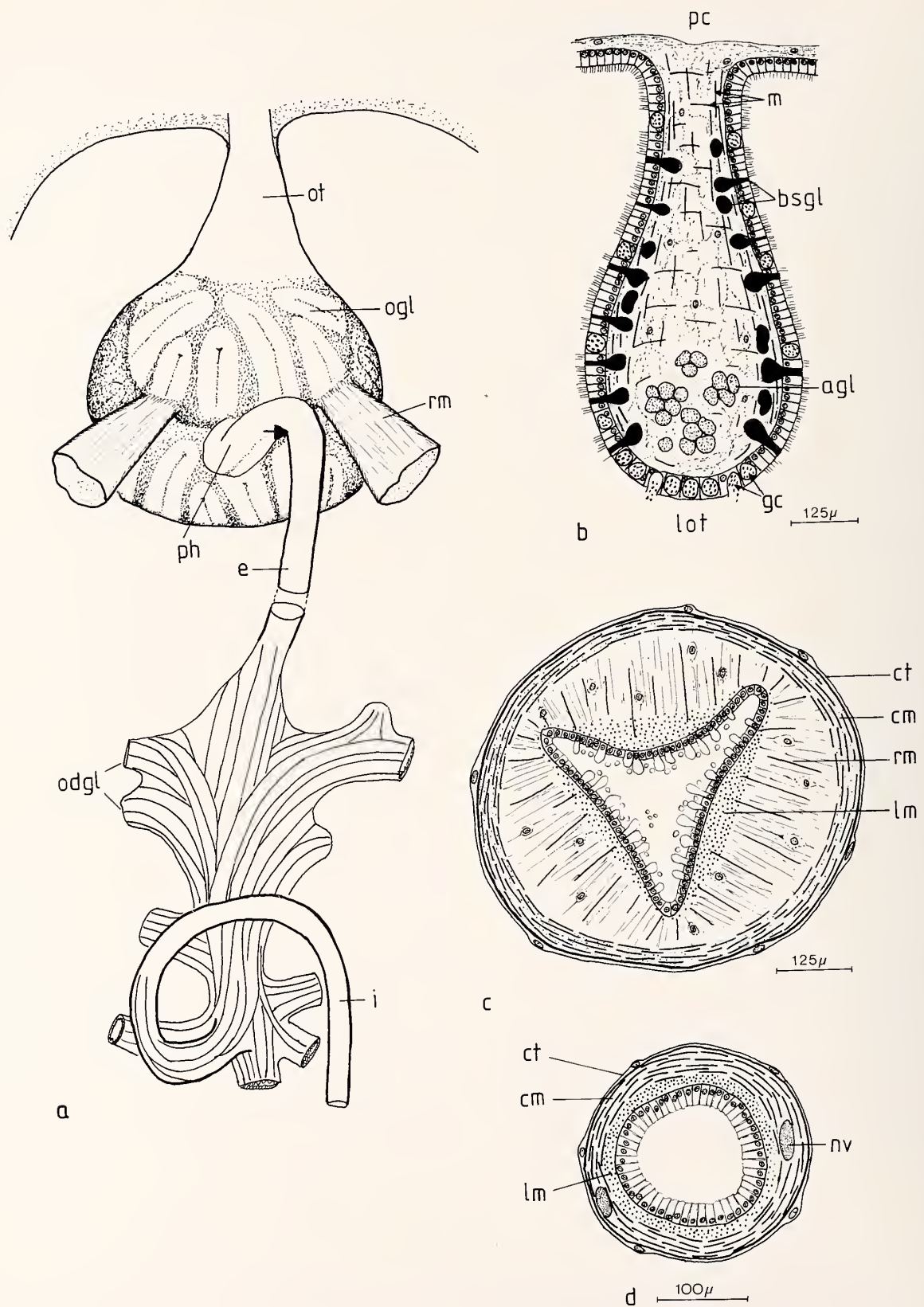
body. Shortly before entering the thick notum tissue, the prostate narrows and forms the ductus ejaculatorius (dej), then passes into the penis (p) without being separated from it. The penis is not armed, and accessory glands were not observed. The vas deferens opens into the vestibulum (Figures 4a, b).

The middle-sized proximal oviduct (Figure 4b: prov) leads ventrally of the female gland to the right side, where it disappears into the gland. Therefore, the bifurcation of the proximal oviduct into the glandular oviduct and the vaginal duct could not be observed macroscopically.

The vaginal duct with its two vesicles lies dorsal to the female gland. The white, oval receptaculum seminis (r) and the yellow-brownish, round bursa copulatrix (b) are both stalked. In the smaller specimens the receptacula were smaller than the bursae. From the bursa the vaginal duct leads directly into the vestibulum, thus having a common opening with the vas deferens (Figure 4a).

Histology: In specimen C1, the largest of the three histologically examined animals, the oocytes of the gonad were in the final stage of development. They measured 85 μm in diameter and were full of granular yolk. The nutritional cells were deformed by the large oocytes into elongate structures. The few regions of sperm development were at the margin of the gonad.

The epithelium of the thin preampullar gonoduct (gd) consists of cuboidal cells. Sometimes sperm were found in the lumen of the duct.

The ampulla is coated with a pavement epithelium, except near the openings of the pre- and postampullar duct, where cuboidal cells predominate. The ampullae of all examined specimens were filled with autosperm, which were distributed irregularly, not lined up (Figure 5d).

The muscular postampullar duct (pogd) is lined with a cuboidal epithelium with long cilia. The duct arises at the distal end of the ampulla and is separated from it by a small septum. At the bifurcation into the vas deferens and proximal oviduct there is a chamberlike enlargement, which is characterized by folds with long cilia.

At the beginning of the vas deferens of C1, some sperm were observed in the lumen. The transition of the proximal vas deferens (which is lined by cuboidal ciliated cells) into the glandular prostate is abrupt. The glandular cells of the prostate are high and rather slender. They are full of acidophilic grana (azan: red). Small apical nuclei indicate that there are supporting cells between the secretory cells (according to SCHMEKEL, 1971). Toward the distal end of the prostate the secretory cells become smaller and disappear at the transition into the ductus ejaculatorius, where cuboidal ciliated cells, thickly underlaid by muscle fibers, dominate.

The penis is a thin, muscular tube forming the end of the ductus ejaculatorius.

The proximal oviduct and its bifurcation in the glandular oviduct and vaginal duct are lined by a cuboidal ciliated epithelium. In the proximal oviduct, spermatozoa were found, single or arranged in groups (Figure 5b: arrows).

The high columnar secretory cells of the membrane gland are densely filled with grana, whereas the contents of the columnar cells of the ripe mucous gland stain homogeneously. The distal part of the mucous gland is formed by a highly folded epithelium of lower, columnar cells, with basophilic granular contents. This seems to be the "adhesive region." In specimen C1, an area was observed with cells that had large, optically empty vacuoles within the cytoplasm (Figure 5a). These seem to be "old" mucous cells that had secreted their contents, indicating that the animal must have had an oviposition.

The vaginal duct is coated with a cuboidal, ciliated epithelium and covered with a layer of muscle fibers at the distal part in particular.

The receptaculum is filled with sperm, the heads of which face the coating epithelium. The tails of the sperm are arranged in lines toward the stalk of the receptaculum (Figure 5e).

The coating of the bursa copulatrix in specimen C1 is a pavement epithelium with large but flat nuclei. Only around the stalk does the epithelium consist of cuboidal cells, which still might have secretory function (see SCHMEKEL, 1971). The lumen is filled with aggregates of more or less dissolved sperm and prostatic secretion granula (Figure 5g). Occasionally oocyte-like cells were found (Figure 5f).

The vestibulum is lined with a columnar ciliated epithelium with mucus-secreting goblet cells between them. The surrounding tissue is interwoven with transverse and longitudinal muscle fibers.

Only spermiogenesis could be observed in the smaller specimens. The development of the prostate secretion was not so advanced as in specimen C1: in smaller specimens,

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Figure 3

Anatomy and histology of the digestive tract. a. Digestive tract seen from the dorsal side; arrow, position of the central nerve ring; the internal septa of the oral glands are indicated. b. Cross section of a septum of the oral gland; position of the section see Figure 2f. c. Cross section of the pharynx. d. Cross section of the esophagus. Key: agl, acidophilic glands; bsgl, basophilic subepithelial glands; gc, goblet cells; ct, connective tissue; e, esophagus; i, intestine; lot, lumen of oral tube; (c,l,r)m, (circular, longitudinal, radial) muscle fibers; nv, nerve; odgl, opening of the digestive gland; ogl, "oral gland"; ot, oral tube; pc, perivisceral cavity; ph, pharynx; rm, retractor muscle.
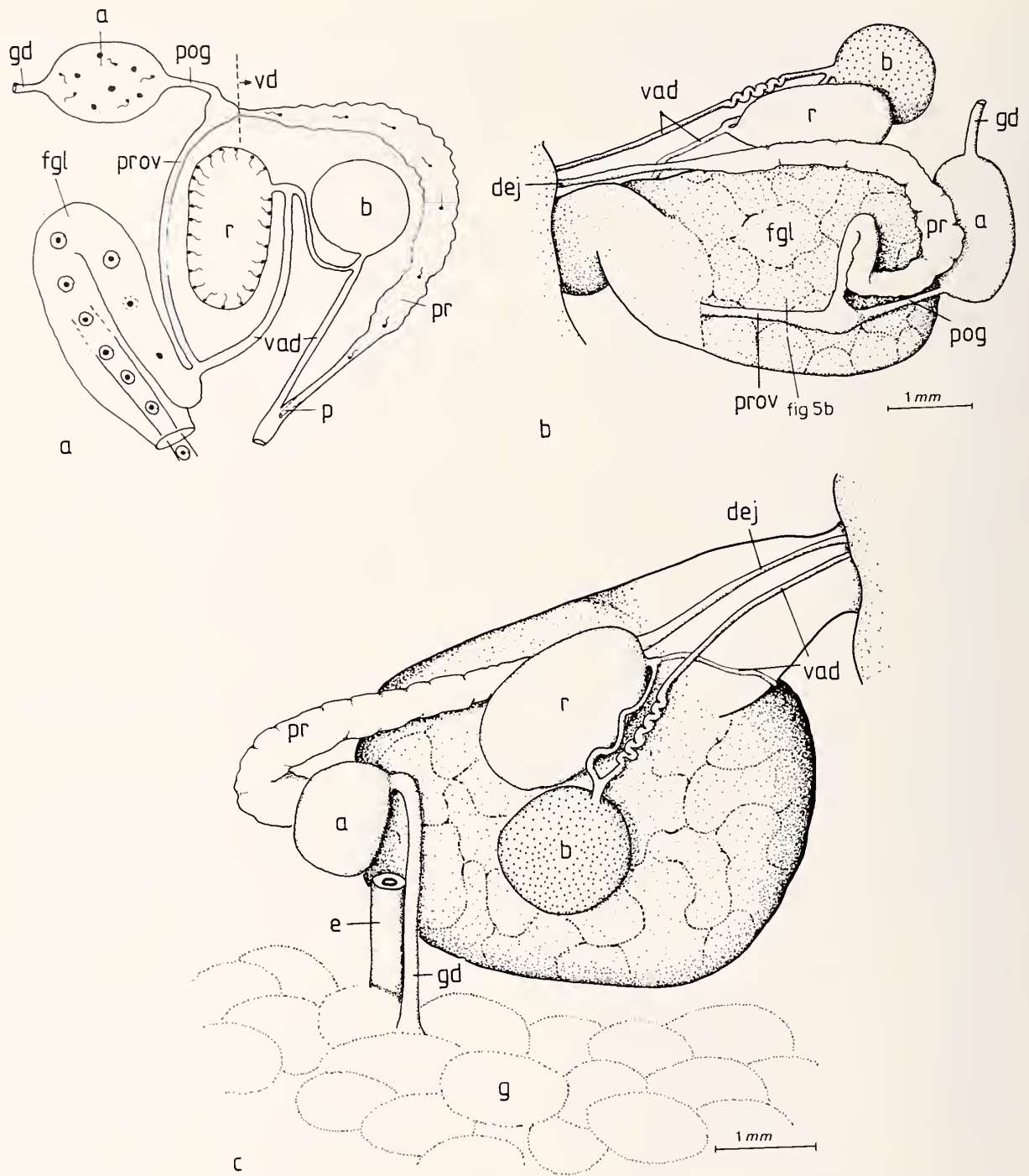
Figure 4

External morphology of genital system. a. General outline. b. *In situ*, seen from the front. c. Same as Figure 4b, dorsal side. Key: a, ampulla; b, bursa copulatrix; dej, ductus ejaculatorius; e, esophagus; fgl, female gland; g, gonad; gd, gonoduct; p, penis; pogd, postampullar gonoduct; pr, prostate; prov, proximal oviduct; r, receptaculum seminis; vad, vaginal duct; vd, vas deferens.

the contents of the cells did not stain red, but instead a bluish-gray. Also, no spermatozoa were found in the proximal oviduct.

Contrary to that of the large specimen C1, the mucous gland in the small specimens contained a great number of immature mucous cells, their contents not being of homogeneous but of granular consistency. No sperm were found in the receptacula seminis. The epithelium of the bursa consisted mainly of apocrine secretory cells (Figure 5c). A pavement epithelium, as found in C1, was not seen; this feature probably depends on the functional phase of the bursa.

Nervous System and Sensory Organs

Morphology: The central nerve ring is placed at the proximal part of the esophagus (Figures 3a: arrow; 6a). The cerebropleural ganglia are close to each other dorsally and connect by the visceral loop (vl) ventrally. All ganglia of this visceral loop are fused with the brain.

The pedal ganglia are placed close to the cerebropleural complex. The thick pedal commissure (pc) lies between the buccal commissure (bc) and the visceral loop.

The two buccal ganglia are placed slightly asymmetrically at the left side of the buccal commissure, which is as short as the other commissures.

In the following list, all nerves originating from the cerebropleural complex are designated with a C, those originating from the pedal ganglia with a P and those branching from the visceral loop with a V. Nerves lying symmetrically on both sides and innervating the same organs are marked by a +.

C1+ Rhinophoral nerve; the proximal part of it forms the bulbous rhinophoral ganglion.

C2+ This nerve runs along the oral tube to the oral tentacles.

C3+ Optic nerve; it is very thin and shows no sign of pigmentation in *Phyllidia pulitzeri*.

C4+ This nerve runs to the rhinophores, and branches several times in the notum; it possibly innervates the rhinophore sheaths and the anterior dorsal part of the notum.

C5 This branch of the right C4 runs ventrally into the notum and could not further be observed.

C6+ This stout nerve leads into the retractor muscles, ramifies, and among other organs innervates the muscle fibers of the oral gland.

C7+ Nervus pallialis posterior (HOFFMANN, 1939); it runs backward along the lateral side of the mantle, occasionally giving off nerves leading to the kidney and heart.

C8 This thin, unpaired nerve lies in the connective tissue of the kidney, innervating the latter and leading to the genital apparatus.

C9 This nerve leads ventrally under the oral glandular mass and disappears into the foot.

P1+ This single pedal nerve is very thick, runs along the posterior part of the club-shaped oral tube to the ventral side, and leads caudally under the visceral mass. It disappears into the tight tissue of the foot in the posterior third of the body.

P2, P3 These two nerves are branches of the right pedal nerve (P1), and innervate the genital organs from the ventral side.

V1 Visceral nerve; branching off from the visceral loop.

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Figure 5

Histology of the genital apparatus. a. "Old" mucous cells of the mucous gland; May-Grünwald/Giemsa; scale 50 μm. b. Cross section of proximal oviduct: for position of the section see Figure 4b; arrows, sperm—note that their heads are turned toward the wall; May-Grünwald/Giemsa; scale 5 μm. c. Apocrine secretory epithelium of bursa copulatrix of small specimen L1; azan; scale 5 μm. d. Section of ampulla, with cuboidal epithelium; May-Grünwald/Giemsa; scale 10 μm. e. Specimen C1, section of the epithelium of the receptaculum seminis; the heads of the sperm are oriented toward the wall; May-Grünwald/Giemsa; scale 5 μm. f. Oocyte-like cell (oo) in the lumen of the bursa; arrow, epithelium of bursa; May-Grünwald/Giemsa; scale 5 μm. g. An aggregation of sperm and prostatic secretion granula; arrows, epithelium of bursa; May-Grünwald/Giemsa; scale 5 μm. Key: b, bursa copulatrix; dgl, digestive gland; lb, lumen of bursa; mgl, membrane gland; mugl, mucous gland; ogl, oral gland; oo, oocyte-like cells; r, receptaculum seminis; vad, vaginal duct.

Figure 6

a. Central nerve ring; dorsal side; at the transition of pharynx (ph), coming from ventrad, into esophagus (e); the esophagus is cut before it passes the nerve ring from ventrad. Key: bg, buccal ganglion; cpg, cerebropleural ganglion; pc, pedal commissure; pg, pedal ganglion; rhg, rhinophoral ganglion; vl, visceral loop. b. General outline of the excretory system. Key: ne, nephridium; ngl, gland in the nephridium; orpd, opening of the renopericardial duct into the nephridium; peep, pericardial epithelium; rpd, renopericardial duct; sy, syrinx; u, ureter; v, digestive gland and gonad covered by the nephridium. c. Epithelia and their position. The four types of glands: agl, acidophilic subepithelial glands (4); bsgl, basophilic subepithelial glands which stain granularly (3); gc, goblet cells (1); hgl, basophilic subepithelial glands which stain homogeneously (2); mu, "crystallized" mucus.
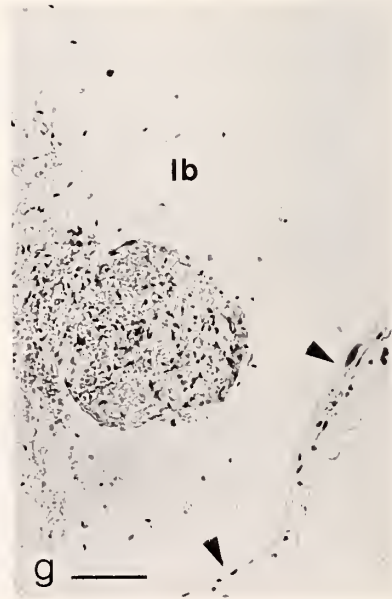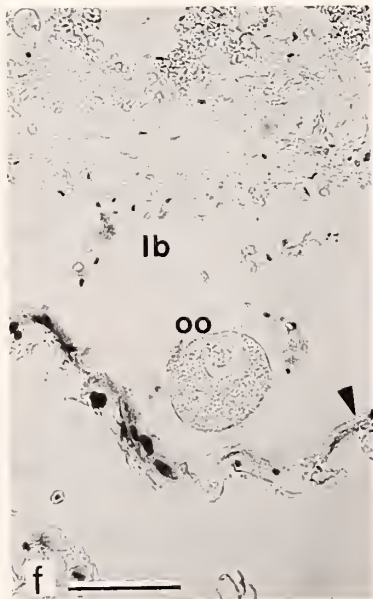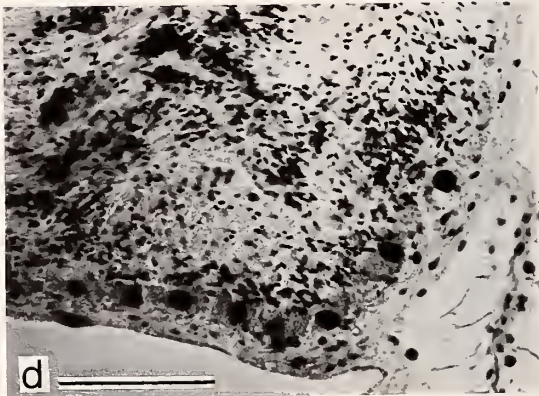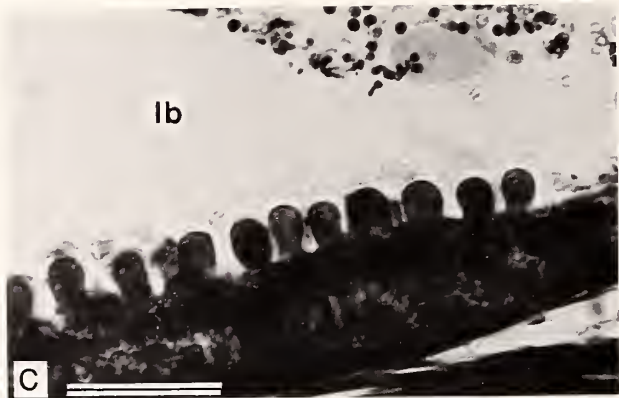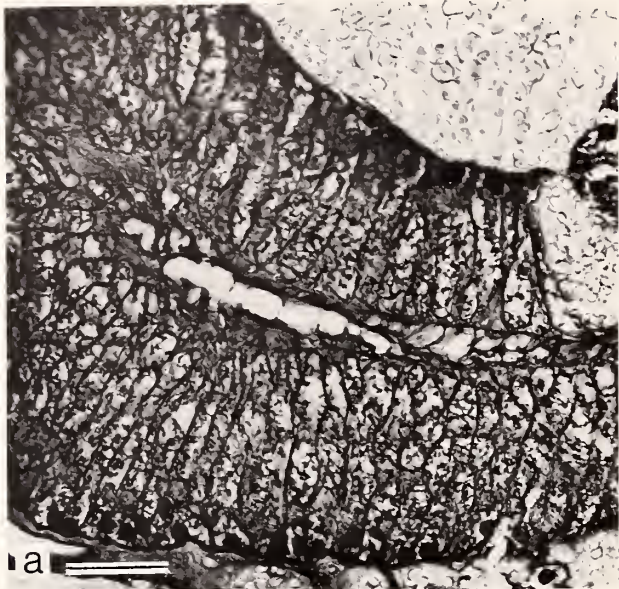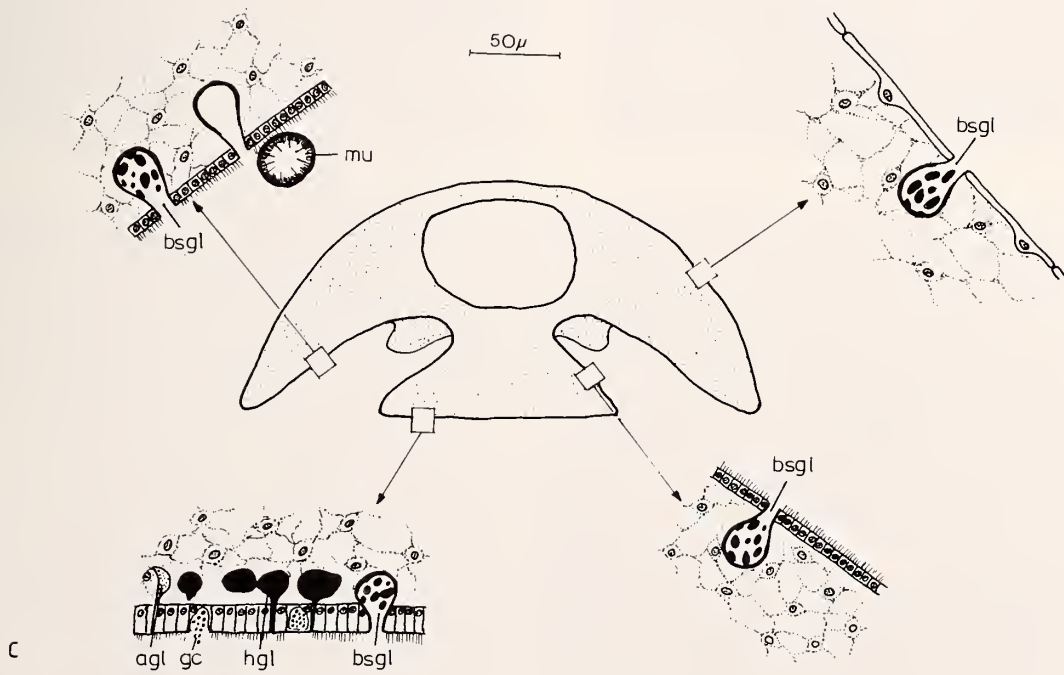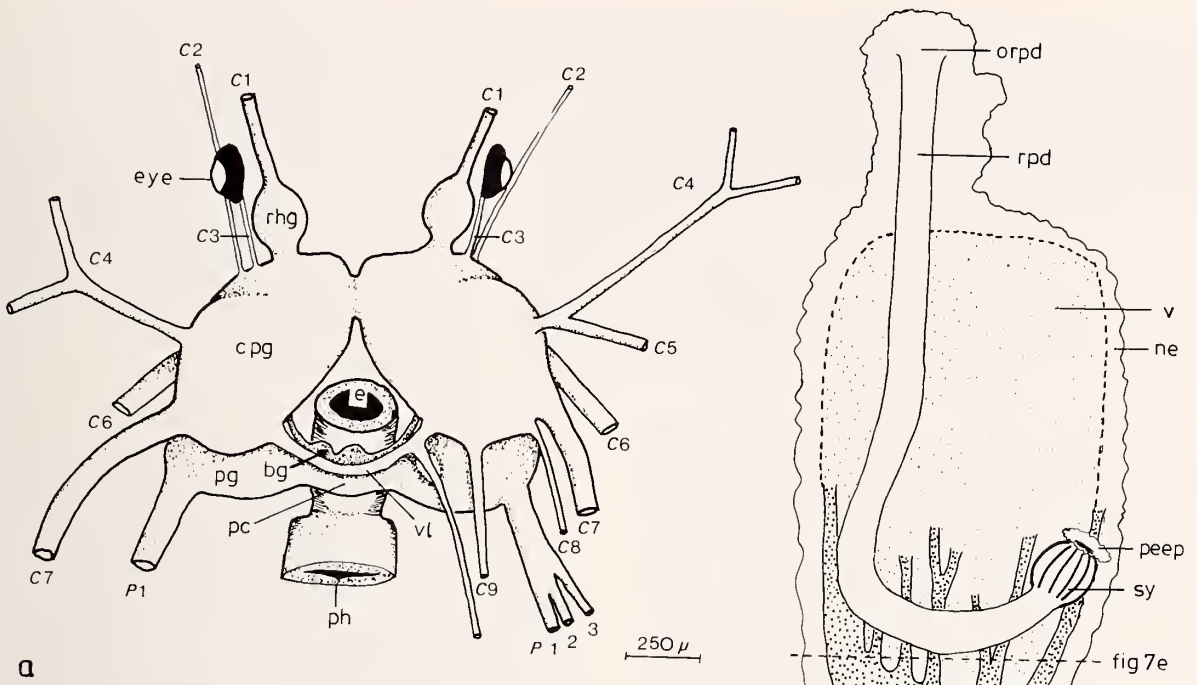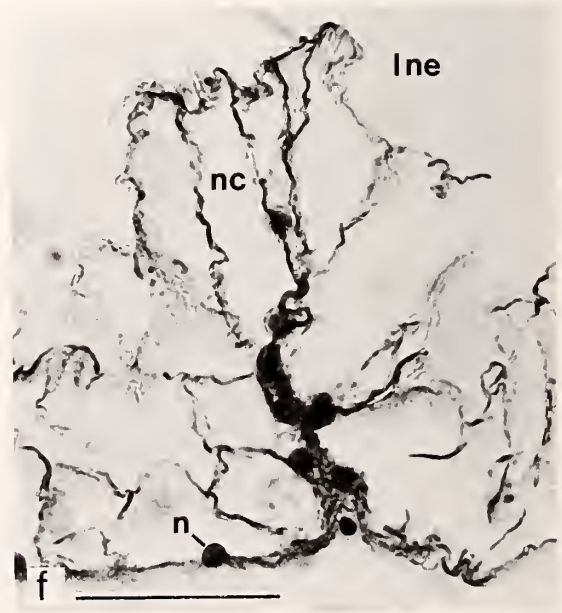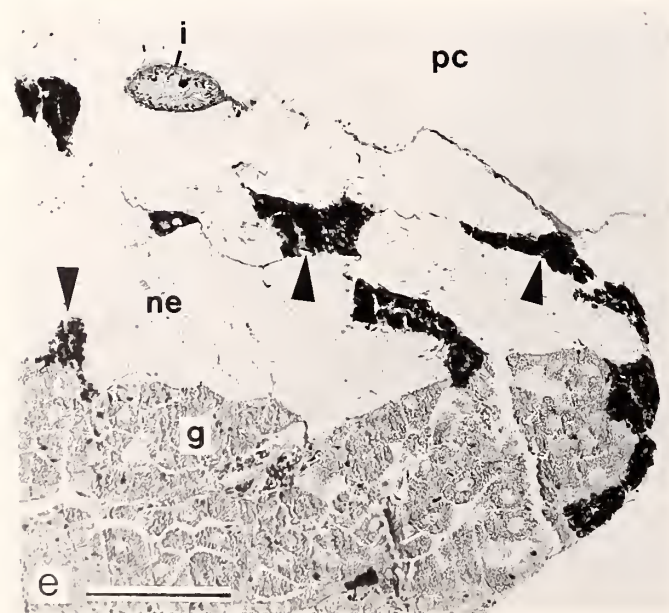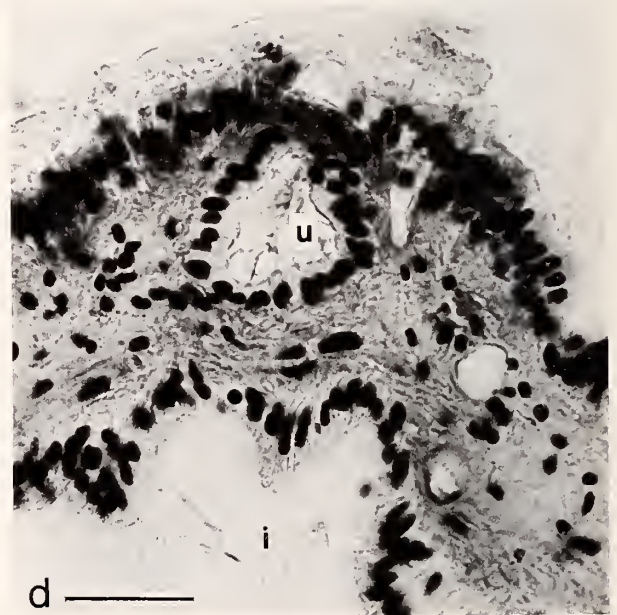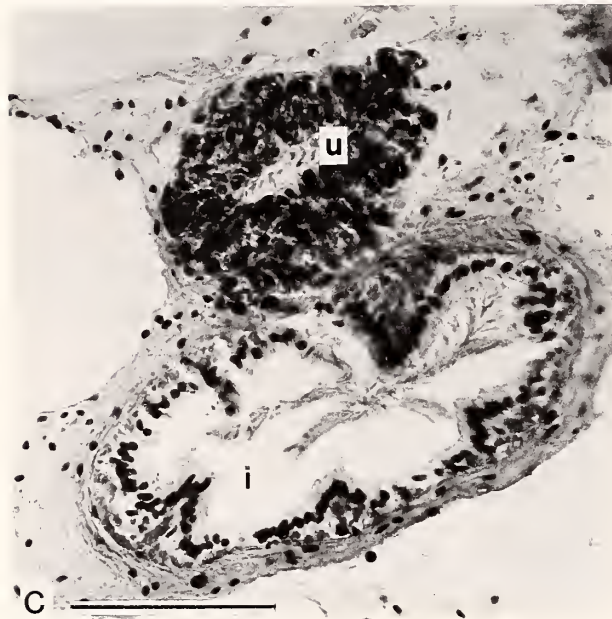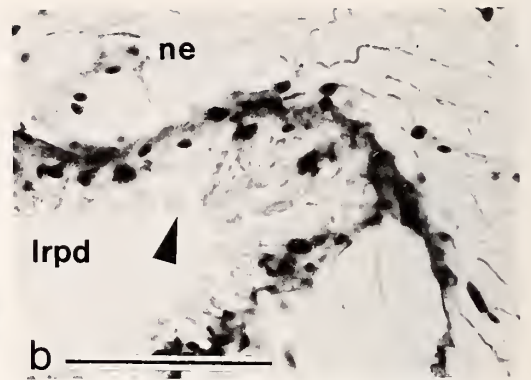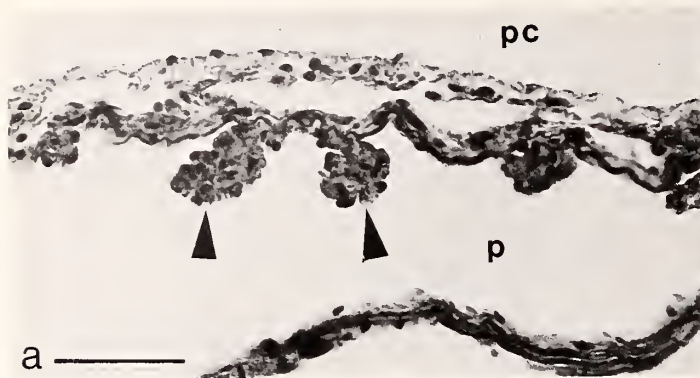
Histology: As mentioned above, two nerves run along the esophagus in the layer of the longitudinal muscle fibers (Figure 3d: nv). These nerves originate in the buccal ganglia and end before the esophagus passes into the stomach. Shortly after their origin in the buccal ganglia, a branch of these nerves runs in the opposite direction to the pharynx, where it shows on each side a ganglionic enlargement in the layer of the longitudinal muscles, exactly at the transition of the esophagus into the pharynx.

The epithelium of the rhinophores is composed of high columnar ciliated cells with basally lying nuclei. Gland cells were not detected. The interior part of the rhinophore contains hyaline tissue (HOFFMANN, 1939), spiculae, and small groups of longitudinal muscle fibers. Spiculae were also observed in the lamellae.

The oral tentacles have an epithelium similar to the ventral side of the notum. In the lateral grooves of the tentacles, larger aggregations of perikarya with well stained nuclei, but with little cytoplasm, were found. These aggregations always lie in hollow spaces of the hyaline tissue. They are probably nuclei of sensory cells, although they could not be recognized as such in this preparation (see HOFFMANN, 1939).

The statocyst of C1 has a diameter of approximately 90 μm. It is placed between the pedal and cerebropleural ganglia (Figure 1c). The inner side is lined by a pavement epithelium. Above this epithelium, toward the cerebropleural ganglion, lies the thin neurilemma (Figure 1c: arrows). The statocyst is filled with 15–20 statoliths of oval to globular form. The average size of the statoliths was 6–8 μm. Cilia were not detected by light microscopy.

Excretory System

Anatomy: The excretory system (Figure 6b) communicates with the pericardium by the so-called syrinx (sy), which lies ventral to the pericardium at the right side. From there, the renopericardial duct (rpd) leads foward under the pericardium to the region of the blood gland, where it enters the nephridium (ne) from the dorsal side.

The nephridium covers the gonad laterally and dorsally and also reaches anteriorly between the viscera. The ramified appearance of the nephridium is caused by the branched vessels in the wall, starting with the aorta posterior.

The ureter originates from the caudal end of the nephridium and opens to the outside on the right side at the base of the anal papilla.

Histology: The wall of the syrinx, a thick layer of longitudinally plicated tissue, is covered at the inner side with a cuboidal epithelium with long cilia. At the transition from syrinx to the renopericardial duct, the cuboidal cells are replaced by apocrine secreting cells (Figure 7b: arrows). In the posterior part of the body, the renopericardial duct is submerged into the nephridium, but in the anterior part it lies dorsal of the nephridium.

The lamellae and folds of the nephridial tissue are mainly formed by the ventral wall of the nephridium. In the posterior part they divide the nephridium into chamberlike areas (Figure 7e). In the folds a thin layer of tissue, with blood vessels in between, separates the cells from each side.

The nephrocytes are large cells with large, nonstaining spaces (Figure 7f).

At the posterior part of the specimen a glandular complex that lies mainly dorsally and laterally on the visceral mass was observed (Figure 6b: ngl). From there, tubuli with a unistriate glandular epithelium and lying in the nephridial tissue reach anteriorly. In specimen L1 these ducts were missing.

The gland is connected with the proximal ureter, where the same large secreting cells of the glandular mass are present between small epithelial cells (Figure 7c: u). The submerged, secreting cells have basal nuclei, whereas the nuclei of the epithelial cells lie apically. Near the end of the ureter the secreting cells are replaced by small ciliated, cuboidal cells (Figure 7d).

Pericardial glands (Figure 7a) which project into the lumen of the pericardium are attached to the anterior dorsal part of the pericardial wall.

Histology of the Epidermis

Figure 6c shows a general outline of the epidermis.

Dorsal notum epithelium: The epithelium is formed by large but flat cells (pavement epithelium) with submerged nuclei. Cilia were not observed. Some basophilic, submerged secretory cells (bsgl) are scattered all over the dorsal side. The contents of these secreting cells have a

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Figure 7

Anatomy and histology of excretory system. a. Section of the dorsal pericardial epithelium with pericardial glands (arrows); hemalaun/lightgreen; scale 5 μm. b. Cross section of renopericardial duct; note the apocrine secretory cells (arrow); hemalaun/lightgreen; scale 10 μm. c. Proximal ureter with secretory cells; for position of section see Figure 6b; May-Grünwald/Giemsa; scale 10 μm. d. Distal ureter; May-Grünwald/Giemsa; scale 5 μm. e. Glandular ducts lying in the nephridial tissue (arrows); for position of the section see Figure 6b; May-Grünwald/Giemsa; scale 25 μm. f. Nephrocytes (nc) of a septum; hemalaun/lightgreen; scale 5 μm. Key: g, gonad; i, intestine; lne, lumen of the nephridium; lrpd, lumen of the renopericardial duct (rpd); n, nucleus; nc, nephrocytes; ne, nephridial tissue; pc, perivisceral cavity; pe, perciardium; u, ureter.

granular appearance. Sometimes the contents evidently had been secreted (mu), and only the lining of the epithelium was stained (dark violet: May-Grünwald/Giemsa). Submerged acidophilic glands (agl) were occasionally found.

Ventral notum epithelium: This epithelium consists of cuboidal ciliated cells. The submerged basophilic glands, mentioned above, are abundant here. Acidophilic glands are not present.

Dorsal foot epithelium: This appears to be the same as the ventral notum epithelium; only basophilic glands are represented.

Ventral foot epithelium: Columnar ciliated cells with basal nuclei dominate.

Four different types of glands are to be found in the ventral foot, and, of these, Types 1 and 2 are glands confined to the foot epithelium.

Type 1: goblet cells (gc) with basophilic granular contents interspersed in the epithelium (see also digestive tract: oral tube and oral gland);

Type 2: subepithelial basophilic glands, with contents stained homogeneously (hgl);

Type 3: subepithelial, granularly stained, basophilic glands;

Type 4: subepithelial acidophilic glands, especially at the margin of the foot.

DISCUSSION

External Morphology

The external appearance of the specimens found in Khalkidhiki and Malta agrees with that of the animals photographed (1974a; animals from Portofino) and described by Barletta (1974a, b) and by Schmekel & Portmann (1982, animals from Capo Miseno and Ponza).

Digestive System

In accordance with Hoffmann (1939) I regard the glandular complex at the opening of the mouth as the foot gland and not as the labial gland, because it lies mainly ventrally and laterally of the vestibulum, not dorsally.

Bergh (1868–69) shows drawings of cross sections through the distal oral tube of *Phyllidia pustulosa* Cuvier, 1804, and *P. varicosa* Lamarck, 1801. These clearly show that the oral tube has inner folds in addition to the lobed or papillate glands at the outside of the posterior oral tube. These outer glands are missing in *P. pulitzeri*. Bergh observed these inner folds in other species too, but did not describe them in detail. Bergh (1889) also mentioned in the first description of *Phyllidiopsis striata* foldlike features in the enlarged part of the oral tube. Because of the lack of anatomical examinations of the outer and inner oral glands and the total absence of histological examinations, possible homologies among different genera of the family Phyllidiidae cannot be discussed.

An "intrabulbous" part of the pharynx, such as observed by Bergh (1868–69) in several species (*Phyllidia varicosa, P. nobilis* Bergh, 1868–69), is not present. The pharynx shows some pecularities in lacking features typical for other groups of the Opisthobranchia: no labial disc or other armament is present at the transition from the oral tube to the pharynx, and radula, mandible, and salivary glands are absent.

A stomach completely separate from the digestive gland, as possessed by other Doridacea, does not exist within the Phyllidiidae. The dorsal part of the central lumen of the digestive tract, where the openings of esophagus and intestine are located, is part of the stomach, whereas the ventral part, with the openings into the digestive gland, seems to represent the central collecting cavity of the digestive gland (see Hoffmann, 1939). The digestive gland corresponds to the holohepatic type. A caecum is absent. No cuticular structures could be found in the entire digestive system.

Genital Apparatus

The anatomy of the genital apparatus of the specimens examined in this study agrees with the description given by Schmekel & Portmann (1982). All examined specimens were sexually mature. The largest of the three histologically examined specimens (C1) seemed to be on the verge of oviposition. This is indicated by the ripe eggs of the gonad, the spermiogenesis restricted to the glandular margin, and the stout membrane gland. No eggs were found in the ampulla or in the distal parts of the genital ducts. This was to be expected, because eggs are transported through the gonoduct and oviduct only during oviposition (Schmekel, 1971).

The smallest of the specimens examined histologically (L1) was in the protandrous phase. Eggs were not yet developed.

Whether the sperm in the proximal oviduct of the larger specimen were autosperm or not, could not be determined.

The structure and position of the membrane gland and the mucous gland are as in other Doridacea (see Schmekel, 1970, 1971). The large mucous gland envelops the tightly coiled membrane gland.

The size of the receptaculum seminis in relation to the size of the bursa probably depends on the quantity of sperm present in the receptaculum. In accordance with this, the empty receptaculum of L1 is small in relation to the bursa, whereas the receptaculum of C1, which was filled with sperm, was larger than the bursa. In the bursa, a dissolution of sperm and of the prostate secretion takes place (Schmekel, 1971).

Nervous System

The enumeration of the nerves and the records of the innervations of organs are still incomplete. To gain a more

accurate conception of innervation more material is necessary.

In some phyllidiids the connectives of the buccal ganglia are very long (see IHERING [1877] for *Phyllidia varicosa;* RISBEC [1956] for *P. honloni* Risbec, 1956; BOUCHET [1977] for *Phyllidiopsis gynenopla* Bouchet, 1977) and the buccal ganglia lie near the stomach. In others they are short (see RISBEC [1956] for *Phyllidia sereni* Risbec, 1956; MARCUS & MARCUS [1970b] for *Phyllidia tula* Marcus & Marcus, 1970) as in *Phyllidia pulitzeri.*

Several authors describe gastro-esophageal ganglia lying near the buccal ganglia (IHERING, 1877; RISBEC, 1928, 1956; MARCUS & MARCUS, 1970a; EDMUNDS, 1972). It may be possible that the ganglionic enlargements of the nerves in the pharyngeal layer of the longitudinal muscle fibers represent the gastro-esophageal ganglia in *Phyllidia pulitzeri.* This would mean that in this species these ganglia are only detectable by histological examinations.

Excretory System

A sphincter muscle around the opening of the syrinx in the pericardium, as described by HANCOCK (1864) for some dorids, is absent in *Phyllidia pulitzeri.*

The renopericardial duct in other dorids is described as lying ventral to the nephridium (see SCHMEKEL & PORTMANN, 1982). In *Phyllidia pulitzeri* it is submerged into the nephridium and is very long as compared with the renopericardial ducts of other Doridacea.

The function of the great glandular complex in the posterior part of the visceral mass is not clear. Whether this gland also exists in other dorids has yet to be examined. BABA (1937) describes in *Okadaia* an accessory renal gland that is separated from the nephridium. This accessory gland opens with a duct into the middle of the ureter. Further investigations must be made before possible homologies can be discussed.

Epidermis

Whether the glands are single glandular cells or multicellular glands could not be determined in the present study.

The basophilic granular type of gland (type 3) seems to be responsible for the secretion of the mucus that "crystallizes" to small globules during fixation with formaldehyde. Sometimes these globular structures could be found even within the glands. Strangely enough, this phenomenon is only mentioned once in the literature (ELIOT, 1910, for *Phyllidiopsis carinata* Eliot, 1910), although Schmekel's specimen from Ponza and also that described by PRUVOT-FOL (1962) have this "crystallized" mucus. The existence of the crystallized mucus apparently depends on the use of certain fixatives. In the presence of acetic acid, which is part of Bouin's fluid and nearly all staining fluids, the globules dissolve. Whether this mucus is identical with the mucus in *Phyllidia varicosa,* described by JOHANNES (1963), is not clear. According to him this mucus, having

a pH of approximately 7, can be secreted in great quantities within a few seconds, and has a poisonous effect on many invertebrates and vertebrates.

Comparing the histological features of the oral gland and the ventral foot epithelium it is remarkable that, except for the basophilic granular type which is absent in the oral gland, both epithelia have the same gland types.

Taxonomic Remarks on the Mediterranean Species of *Phyllidia*

Three species of the genus *Phyllidia* are known from the Mediterranean at present: *P. rolandiae* Pruvot-Fol, 1951; *P. aurata* Pruvot-Fol, 1952; and *P. (Phyllidiopsis ?) pulitzeri* Pruvot-Fol, 1962.

PRUVOT-FOL (1952) was in doubt as to whether *Phyllidia rolandiae* and *P. aurata* should be placed within the genus *Phyllidia.* Although they differed in some features (*e.g.,* they lack the black color so typical for almost all species of the family Phyllidiidae), she did not want to erect a new genus based on only two specimens. As she did not dissect *P. pulitzeri,* she was uncertain as to which genus it belonged. The present study settles this problem: similar to the descriptions of the type species of *Phyllidia* (*P. varicosa* Lamarck, 1801, described by BERGH, 1868–69), *P. pulitzeri* has a posterior club-shaped oral tube. Furthermore, there is no oral gland lying free ventral to the oral tube; therefore, it cannot belong to the genus *Phyllidiopsis.*

When the descriptions of *Phyllidia rolandiae* and *P. pulitzeri* are compared, no differences of taxonomic value can be detected. Unfortunately, information given on the same specimens differs in subsequent publications, and the first descriptions of these species were inadequate. Many features of *P. rolandiae* can only be inferred from PRUVOT-FOL's (1952) description of *P. aurata.* The gills of *P. aurata* are said to be "moins saillantes"; hence, those of *P. rolandiae* have to be bigger, similar to those of *P. pulitzeri.* Information on features such as size is not sufficiently reliable, especially in organs where different kinds of fixation may cause variability. The same applies to the form and size of the tubercles as demonstrated for *P. pulitzeri* in the present study.

The only differences between *Phyllidia rolandiae* and *P. pulitzeri* seem to be the shape of the surface of the tubercles ("bosselés" in *P. rolandiae* [PRUVOT-FOL, 1951: 37], smooth in *P. pulitzeri*) and the isolated gland near the posterior part of the oral tube in *P. rolandiae,* which is lacking in *P. pulitzeri.* In the first description of *P. pulitzeri,* PRUVOT-FOL (1962:568–569) differentiates between large tubercles, composed of five or six translucent "spherules" and smaller tubercles, composed of one, two, or three "spherules." She further states "Elles tiennent solidement au tégument; et ceci, en plus de la disposition plus ou moins régulière sur le manteau, exclut l'idée d'un artefact, qu'il fallut tout d'abord écarter, à cause de leur forme sphérique insolite." What she describes here very

likely is the mucus that, indeed, can hardly be removed from the surface, being partly crystallized in the epithelium. This assumption is supported by the fact that, in Pruvot-Fol's figure of *P. pulitzeri*, some globules are drawn on the ventral side of the notum.

Unfortunately a re-examination of the holotypes of the three Mediterranean species is not possible, as the holotypes of *Phyllidia rolandiae* and *P. pulitzeri* are considered to be lost (personal communication from Dr. P. Bouchet, Paris) and the holotype of *P. aurata*, located at the Muséum National d'Histoire Naturelle in Paris, is totally dissected, so that no organs are left except the notum.

The examination of this notum revealed that *Phyllidia aurata* is clearly distinguishable by the granular appearance of its tubercles. This granular surface is caused by the spiculae with their outwardly pointing ends. The tubercles of *P. aurata* are arranged in lines (not mentioned by Pruvot-Fol, 1952), as can be seen in the fixed holotype, although this is difficult to make out, as it is in the fixed specimens of *P. pulitzeri*.

The possibility cannot be excluded that *Phyllidia rolandiae* is identical to one of the other two Mediterranean species of the genus. Most of the few features known (color not known, tubercles "bosselés," gills larger than in *P. aurata*) cannot be used to characterize a species. As to the only feature of importance, the "couche glandulaire," the possibility of confusion with the blood gland must be considered. *Phyllidia rolandiae* also has a small anal tubercle, which is thought to be absent in *P. aurata* but is present in *P. pulitzeri*. In my specimens of *P. pulitzeri*, however, this feature shows great variability. Therefore, at present *P. rolandiae* cannot be distinguished from the other species. Certainly, the "*P. rolandiae*" mentioned by Barash & Danin (1982) is a *P. pulitzeri*. Thus, all evidence seems to indicate that *P. rolandiae* has to be regarded as a *nomen dubium*.

ACKNOWLEDGMENTS

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